Available online at www.sciencedirect.com

ScienceDirect

C. R. Biologies 332 (2009) 25–33

COMPTES RENDUS BIOLOGIES

http://france.elsevier.com/direct/CRASS3/

Biochemistry / Biochimie

Nutritional and biological qualities of the ripened beans of *Canavalia maritima* from the coastal sand dunes of India

B. Bhagya [a], K.R. Sridhar [a,*], N.S. Raviraja [b], C.-C. Young [c], A.B. Arun [c]

[a] *Microbiology and Biotechnology, Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore 574 199, Karnataka, India*

[b] *Department of Oncology, Montefiore Medical Center/Albert Einstein Cancer Center, Hofheimer Bldg. 413, 111 E. 210th Street, Bronx, NY 10467, USA*

[c] *College of Agriculture and Natural Resources, Department of Soil Environmental Science, National Chung Hsing University, 250 Kuo-Khang Road, Taichung, Taiwan 40227, Republic of China*

Received 11 August 2008; accepted after revision 16 September 2008

Available online 24 December 2008

Presented by Philippe Morat

Abstract

Raw and pressure-cooked ripened beans of *Canavalia maritima* were assessed for nutritional quality. The beans possess high protein, carbohydrate, fiber and energy contents. Potassium, magnesium, zinc and manganese of the raw and cooked beans meet NRC/NAS recommended pattern for infants. The essential amino acids (threonine, valine, isoleucine, leucine, tyrosine/phenylalanine and lysine) in raw and cooked ripened beans fulfill the FAO/WHO/UNU recommended pattern for adults. Oleic acid in raw beans and linolenic acid in cooked beans were highest and linoleic and arachidonic acids were confined to raw beans. Cooking lowered the total phenolics, while tannins were negligible and devoid of orthodihydric phenols and trypsin inhibitors. Hemagglutinating activity decreased up to 50% in cooked beans. Rats fed with a pressure-cooked bean diet showed significant elevation of all growth and nitrogen balance parameters ($P < 0.05$) than the rats which received the raw bean diet. The low protein quality of beans warrants appropriate thermal processing to eliminate antinutritional factors. ***To cite this article: B. Bhagya et al., C. R. Biologies 332 (2009).***

Keywords: *Canavalia maritima*; Ripened beans; Minerals; Amino acids; Fatty acids; Antinutritional factors; Protein quality

1. Introduction

Pulses meet only about 20% of protein requirement in developing countries [1] facing the scarcity of animal protein, the exploitation of wild legumes is necessary to combat protein malnutrition [2]. Investigations on the nutritional features of African wild legumes revealed the potential usefulness as food [3–7]. Many wild legumes, although they contain antinutritional factors, possess adequate amount of proteins, essential amino acids, essential fatty acids and vitamins. About 28 wild legumes are known to be consumed as food by tribal sects in India [8,9]. Among them, the genera *Canavalia* comprises of four subgenera and 51 species distributed in tropical and subtropical biomes [10]. *Canavalia maritima* Thouars has been widely distributed in coastal sand dunes of the southwest coast of India [11]. The

* Corresponding author.
E-mail address: sirikr@yahoo.com (K.R. Sridhar).

1631-0691/$ – see front matter
doi:10.1016/j.crvi.2008.09.013

dry seeds of *C. maritima* comprise a potential source of proteins and minerals, but the presence of antinutritional factors limits their use [12,13]. The aim of the present study is to evaluate biochemical, nutritional and antinutritional qualities of raw and pressure-cooked ripened beans of *C. maritima* obtained from the sand dunes of southwest coast of India. This study also envisages evaluating protein qualities of raw and cooked ripened beans through growth and nitrogen balance using a rat model.

2. Material and methods

2.1. Materials

Light yellow mature pods of *C. maritima* were harvested during post-monsoon season (November 2002) from the coastal sand dunes of Kanhangad, Kerala, on the southwest coast of India (12°20′N, 75°05′E). They were de-shelled to separate ripened beans. Dry weight of beans, cotyledons and bean coat were determined (dried at 80 °C for 24 h). Fresh beans were divided into 2 sets of which the first set was sun dried. Each bean of the second set was cut into four pieces, pressure-cooked with freshwater (1:3 v/v), and sun dried. Raw and pressure-cooked beans were powdered (Wiley Mill, 30 mesh) and preserved in airtight glass containers.

2.2. Methods

2.2.1. Proximate and mineral analysis

Moisture of bean flour was determined after attaining constant weight at 80 °C. Total nitrogen and the crude protein (N × 6.25) were determined by the micro-Kjeldahl method [14]. Crude lipid, crude fiber, ash, and minerals were assessed based on methods outlined in Association of Official Analytical Chemists Methods [15]. Crude carbohydrate and gross energy were calculated by the procedures outlined by the Müller and Tobin [16] and the Osborne and Voogt methods [17], respectively. The vitamin C was estimated according to Roe [18], while phosphorus content was measured using the ascorbic acid method [19].

2.2.2. Protein fractions and isolation

Protein fractions of the bean flours were extracted based on Basha et al. [20]. To extract the proteins, seed flours (in 100 µg aliquots) were dissolved in 100 µl of buffer (Tris-HCl, 60 m Mol l^{-1}, pH 6.8, 10% w/v; glycerol, 2% w/v; sodium dodecyl sulfate (SDS) and mercaptoethanol, 10% v/v), boiled (2 min at 100 °C), cooled, and bromophenol blue solution (2 µl, 50%, w/v) was added [21]. Protein separation was carried out using 1-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a 5% (w/v) stacking gel and 13.5% (w/v) separating gel [22]. An equal quantity of protein was loaded to the gel, run for 3 h (70 V), and stained with Coomassie brilliant blue R-250.

2.2.3. Amino acid analysis

The method described by Hofmann et al. [23,24] was followed to assess amino acids. Bean flours (15 mg) were hydrolyzed (15 ml 6 N HCl for 4 h at 145 °C), cooled, and HCl was eliminated in a rotoevaporator combined with a diaphragm vacuum pump. An internal standard, trans-4-(aminomethyl)-cyclohexanecarboxylic acid, was added to each sample for quantitative analysis of amino acids. The derivatization consisted of esterification with trifluoroacetylation [25]. Samples were dried using CH_2Cl_2, 12 ml of fresh acidified isopropanol (acetyl chloride, 3 ml + 2-propanol, 12 ml) was added, mixed, heated (110 °C, 1 h), cooled, and filtered through glass fiber paper. The reagent was eliminated in a gentle stream of helium (45 °C) followed by combined evaporation with aliquots of CH_2Cl_2. The residue was acetylated (300 µl of trifluoroacetic anhydride, 12 h, 20 °C) and amino acids were determined using gas chromatography-combustion-isotope ratio mass spectrometer (Hewlett Packard 5890 series II; MAT 252).

2.2.4. Fatty acid analysis

Fatty acid methyl esters (FAMEs) were determined following the method by Garces and Mancha [26]. Bean flours (50 mg) with fatty acid standard (American Oil Chemists Society) (AOCS) were taken in tubes with Teflon-lined caps and methylated with a mixture containing methanol; benzene; 2,2-dimethoxypropane (DMP) and H_2SO_4 (37:20:5:2 v/v). The sample was placed in water bath (80 °C for 2 h) and the mixture was made up to a total volume of 5 ml with heptane, cooled and shaken to separate two phases. One ml upper layer containing FAMEs was injected to gas liquid chromatograph and separated using a glass column (Silar, 10% packed with ethylene glycol succinate, 5% on Supelcoport 80/100 isothermically at 200 °C). The ratio of polyunsaturated and saturated fatty acid (P/S ratio) was calculated.

2.2.5. Antinutritional factors

Total phenolics in the bean flours were determined after extracting in 50% methanol [27]. Tannins were determined by a radial diffusion method using bovine

Table 1
Diet composition (g 100/g).

Ingredients	Basal diet	Casein diet	Test diets	
			Raw bean diet	Cooked bean diet
Cornstarch	80	70	47.3	43.6
Corn oil	10	10	10	10
Non-nutrition cellulose	5	5	5	5
Casein	–	10	–	–
Raw bean flour	–	–	32.7	–
Cooked bean flour	–	–	–	36.4
Salt mixture[a]	4	4	4	4
Vitamin mixture[b]	1	1	1	1

[a] Salt mixture: $CaCO_3$, 78.6 g; $Ca_3C_{12}H_{10}O_{14}\cdot 4H_2O$, 308.3 g; $CaHPO_4\cdot 2H_2O$, 112.8 g; K_2HPO_4, 218.8 g; KCl, 124.7 g; NaCl, 77.1 g; $MgSO_4$, 38.3 g; $MgCO_3$, 35.2 g; $Fe(C_6H_{17}N_3O_7)$, 15.3 g; $MnSO_4\cdot H_2O$, 0.201 g; $CuSO_4\cdot 5H_2O$, 0.078 g; KI, 0.041 g; $AlNH_4(SO_4)_2\cdot 12H_2O$, 0.507 g.

[b] Vitamin mixture: vitamin A, 1000 IU; vitamin D, 100 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; thiamine, 0.5 mg; riboflavin, 1 mg; pyrodoxine, 0.4 mg; pantothenic acid, 4 mg; niacin, 4 mg; choline, 200 mg; inositol, 25 mg; para-aminobenzoinc acid, 10 mg; vitamin B_{12}, 2 µg; biotin, 0.02 mg; folic acid, 0.2 mg; added cellulose to make up to 1 g.

serum albumin for precipitation [28]. Orthodihydric phenols were estimated employing the method by Mahadevan [29]. Trypsin inhibitory activity of bean flour was determined by an enzyme assay [30]. Hemagglutinating activity was carried out using a rabbit erythrocyte suspension [31,32]. Trypsin-treated erythrocytes were suspended in phosphate-buffered saline to have a 2% (v/v) cell suspension. Twofold serial dilutions of crude lectin (50 µl) with the erythrocyte suspension (50 µl) were incubated in microtitre plates (30 min, 30 °C) and examined for hemagglutination. Maximum dilution where agglutination was observed was considered as titre value.

2.2.6. Biological evaluation of protein quality

The experimental protocol has been performed following the approved norms (Ministry of Social Justice and Empowerment, Government of India # 25/1/99–AWD). The Wistar male rats (21-day-old each weighing 30 ± 5 g) were divided randomly into 4 groups each with 5 rats and housed in polypropylene metabolic cages (room temperature, 22 ± 1 °C, 50% relative humidity, 12 h photoperiod). Food and water was provided *ad libitum*. The rats were offered 4 different diets: protein-free diet (as basal diet), casein diet (as standard protein diet), and raw and pressure-cooked bean flour diets (as test diets) (Table 1). The basal diet consisted of cornstarch (80%), corn oil (10%), non-nutritive cellulose (5%), salt mixture (4%), and vitamin mixture (1%). The rat group fed with casein (10%) as a source of protein served as the control. Raw and pressure-cooked bean flours were incorporated into the basal diet at the expense of cornstarch to make up 10% protein. The protein efficiency ratio (PER) and net protein ratio (NPR) were carried out up to 28 days according to the method by Pellet and Young [33]. Food consumption and body weight of rats were assessed at weekly as well as 10-day intervals. The PER, corrected PER, food efficiency ratio (FER) for 4 weeks, NPR for 10 days, and protein retention efficiency (PRE) were calculated [34].

Nitrogen balance studies were performed according to Chick et al. [35]. Twenty adult male rats (weighing 60–68 g) were divided into 4 groups and fed 1 of the 4 diets (protein-free, raw bean, pressure-cooked bean, and casein). The experiment was continued for 14 days (9 days for acclimatization; 5 days for collection). The nitrogen in urine and feces was estimated using the micro-Kjeldahl method [15]. The true digestibility (TD), biological value (BV), and net protein utilization (NPU) were evaluated [36].

2.3. Statistical analysis

Paired *t* test was employed to detect the difference in nutritional and antinutritional composition between raw and pressure-cooked beans. The differences in growth and nitrogen balance in rats fed with raw and cooked bean diet were evaluated by paired *t* test [37].

3. Results

3.1. Bean features, proximal and mineral composition

Whole bean, cotyledon, and bean coat weights ($n = 20$) were 0.19 ± 0.12, 0.1 ± 0.08, and 0.09 ± 0.05 g, respectively. The bean length, width, thickness, and hilum length ($n = 20$) were 1.51 ± 0.21, 0.67 ± 0.14, 0.67 ± 0.06, and 0.41 ± 0.07 cm, respectively. Among proximal features, except for total carbohydrates and energy, the rest were significantly lower in cooked beans than in raw beans ($P < 0.05$) (Table 2). Cooking drastically declined minerals in beans ($P < 0.05$) (Table 3).

3.2. Proteins, amino acid and fatty acid profiles

Total proteins, albumins, globulins and glutelins were higher in raw beans than cooked beans (Table 4). Except for prolamins, the rest of the protein fractions significantly differed between raw and cooked beans ($P < 0.05$). Globulins (13.3–16.6%) constitute the major fractions in raw as well as cooked beans followed by

Table 2
Proximate composition of ripened bean flours of *Canavalia maritima* on dry weight basis.

Component	Ripened beans	
	Raw*	Cooked*
Moisture (%)	8.55 ± 0.39^a	6.34 ± 0.42^b
Crude protein (g 100/g)	30.62 ± 0.62^a	27.47 ± 1.59^b
Crude lipid (g 100/g)	2.26 ± 0.17^a	1.92 ± 0.13^b
Crude fiber (g 100/g)	10.65 ± 1.06^a	6.98 ± 0.41^b
Ash (g 100/g)	3.18 ± 0.08^a	2.40 ± 0.21^b
Crude carbohydrates (g 100/g)	53.29 ± 1.38^a	61.23 ± 1.72^b
Energy value (kJ 100/g)	1490 ± 18.45^a	1558 ± 6.67^b
Vitamin C (mg 100/g)	0.23 ± 0.02^a	0.07 ± 0.01^b

* Values (mean ± SD, $n = 5$) across the columns with different letters are significantly different ($P < 0.05$, t test).

Table 3
Mineral compositions of ripened bean flours of *Canavalia maritima* on dry weight basis (mg 100/g).

Minerals	Ripened beans		NRC/NAS pattern**
	Raw*	Cooked*	
Sodium	53.77 ± 3.33^a	34.60 ± 3.35^b	120–200
Potassium	1028.00 ± 108.31^a	627.67 ± 47.7^b	500–700
Calcium	139.20 ± 6.94^a	91.43 ± 2.25^b	600
Phosphorus	228.29 ± 2.83^a	159.52 ± 6.01^b	500
Magnesium	84.73 ± 5.89^a	78.20 ± 6.82^b	60
Iron	1.39 ± 0.09^a	0.70 ± 0.06^b	10
Copper	0.48 ± 0.004^a	0.27 ± 0.02^b	0.6–0.7
Zinc	11.57 ± 0.71^a	3.48 ± 0.18^b	5
Manganese	1.58 ± 0.35^a	0.31 ± 0.08^b	0.3–1
Selenium	39.45 ± 3.18^a	31.32 ± 1.35^b	–

* Values (mean ± SD, $n = 5$) across the columns with different letters are significantly different ($P < 0.05$, t test).
** NRC/NAS [57] pattern for infants.

Table 4
True proteins and their fractions of ripened bean flours of *Canavalia maritima* on dry weight basis (mg 100/g) (% in parenthesis).

Protein fractions	Ripened beans	
	Raw*	Cooked*
True proteins	27.01 ± 0.21 (100)^a	20.93 ± 0.61 (100)^b
Albumins	7.41 ± 0.27 (27.43)^a	5.04 ± 0.22 (24.08)^b
Globulins	16.61 ± 0.42 (61.5)^a	13.25 ± 0.53 (63.31)^b
Prolamins	0.74 ± 0.02 (2.74)^a	0.55 ± 0.04 (2.63)^a
Glutelins	2.25 ± 0.09 (8.33)^a	2.08 ± 0.03 (9.94)^b

* Values (mean ± SD, $n = 5$) across the columns with different letters are significantly different ($P < 0.05$, t test).

albumins (5–7.4%). The SDS-PAGE of raw beans resulted in four major protein fractions (53.7, 40.36, 34.1 and 16.49 kDa), while three protein fractions in cooked beans (48.59, 26.96 and 9.86 kDa) with smear like unclear bands.

Table 5
Amino acid composition of ripened bean flours of *Canavalia maritima* (g 100/g protein).

Amino acid	Ripened beans		FAO/WHO/UNU pattern[a]
	Raw	Cooked	
Glutamic acid	19.09	10.53	
Aspartic acid	7.36	4.66	
Serine	4.27	1.93	
Threonine	2.28	1.57	0.9
Proline	1.98	1.72	
Alanine	2.10	2.01	
Glycine	1.45	1.42	
Valine	4.22	2.08	1.3
Cystine	ND[d]	ND[d]	
Methionine	0.90	0.84	1.7[b]
Isoleucine	4.24	1.52	1.3
Leucine	4.52	0.26	1.9
Tyrosine	10.31	0.20	
Phenylalanine	4.08	3.09	1.9[c]
Tryptophan	ND[d]	ND[d]	0.5
Lysine	4.26	4.22	1.6
Histidine	ND[d]	ND[d]	1.6
Arginine	ND[d]	ND[d]	

[a] FAO/WHO/UNU amino acids pattern for adults [38].
[b] Methionine + Cystine.
[c] Phenylalanine + Tyrosine.
[d] ND = Not detectable.

Amino acid profile of raw and cooked beans have been compared with Food and Agricultural Organization/World Health Organization/United Nations University (FAO/WHO/UNU) pattern of essential amino acid (EAA) requirement for adults [38] (Table 5). The beans were devoid of arginine, cystine, histidine and tryptophan. The glutamic acid was highest in raw (19.1%) as well cooked beans (10.5%) followed by aspartic acid (7.4 and 4.7%). Phenylalanine and lysine of raw beans were not drastically declined on cooking, while leucine and tyrosine drastically decreased.

The raw beans consist of more unsaturated fatty acids, whereas saturated fatty acids in cooked beans (Table 6). The oleic acid was highest in raw beans, while lauric acid in cooked beans. Among essential fatty acids, linoleic as well as arachidonic acids were confined to raw beans. Cooking increased linolenic acid in beans (0 vs. 7.3 mg/g lipid). The polyunsaturated fatty acids/saturated fatty acids (P/S) ratio was elevated in raw than cooked beans.

3.3. Antinutritional features

Total phenolics and tannins were significantly lowered in cooked beans ($P < 0.05$) (Table 7). Total phenolics decreased to about 50% in beans on cooking. Tannins of beans were negligible and further lowered on

Table 6
Mean fatty acid values (mg/g lipid, $n = 3$) of ripened bean flours of *Canavalia maritima*.

Fatty acid	Ripened beans	
	Raw	Cooked
Saturated fatty acids		
Lauric acid ($C_{12:0}$)	0.44	12.47
Myristic acid ($C_{14:0}$)	0.99	0.33
Pentadecanoic acid ($C_{15:0}$)	0.23	0.28
Palmitic acid ($C_{16:0}$)	2.67	1.15
Heptadecanoic acid ($C_{17:0}$)	ND[a]	2.13
Stearic acid ($C_{18:0}$)	0.07	0.5
Tricosanoic acid ($C_{23:0}$)	ND[a]	0.02
Pentacosanoic acid ($C_{25:0}$)	0.02	ND[a]
Polyunsaturated fatty acids		
Myristoleic acid ($C_{14:1}$)	0.31	0.02
Palmitoleic acid ($C_{16:1}$)	0.51	1.76
Oleic acid ($C_{18:1}$)	6.06	ND[a]
Linoleic acid ($C_{18:2}$)	4.53	ND[a]
Linolenic acid ($C_{18:3}$)	ND[a]	7.28
Eicosenoic acid ($C_{20:1}$)	4.96	ND[a]
Eicosadienoic acid ($C_{20:2}$)	ND[a]	2.12
Arachidonic acid ($C_{20:4}$)	0.001	ND[a]
Eicosapentaenoic acid ($C_{20:5}$)	0.002	0.08
Nervonic acid ($C_{24:1}$)	0.002	ND[a]
Sum of essential fatty acids	**4.531**	**7.28**
Sum of saturated fatty acids	**4.42**	**16.9**
Sum of polyunsaturated fatty acids	**16.4**	**11.3**
***P/S* ratio**[b]	**3.7**	**0.67**

[a] ND = Not detectable.
[b] P/S ratio: ratio of polyunsaturated/saturated fatty acids.

Table 7
Antinutritional components of ripened bean flours of *Canavalia maritima*.

Component	Ripened beans	
	Raw	Cooked
Total phenolics (g/100 g)*	5.480 ± 0.030^a	2.720 ± 0.130^b
Tannins (g/100 g)*	0.051 ± 0.005^a	0.019 ± 0.002^b
Orthodihydric phenols	NP	NP
Trypsin inhibition activity	NP	NP
Hemagglutination activity**	23	13

NP indicates not present.
* Values (mean ± SD, $n = 5$) across the columns with different letters are significantly different ($P < 0.05$, t test).
** Titre value: maximum dilution where agglutination was observed.

cooking, while they were devoid of orthodihydric phenols and trypsin inhibitors. Raw beans exhibited severe agglutination of rabbit erythrocytes and it diminished to about 50% on cooking.

3.4. Protein quality

Results on the growth and nitrogen balance experiments in rats on feeding control and test diets have been compared in Table 8. Pressure-cooked bean diet significantly elevated growth as well as nitrogen balance parameters in rats ($P < 0.05$). Food intake of cooked beans was better than raw beans (117.74 vs. 90.06 g), so also the protein intake (11.77 vs. 9.06 g). Although the gain in body weight on feeding raw or cooked bean diets was lower than for the casein diet, the cooked bean diet elevated the body weight more than the raw bean diet (2.19 vs. 0.56 g for 10 days). The overall growth and nitrogen balance parameters of cooked beans were lower than casein diet, but it was higher than the raw bean diet.

4. Discussion

Dry weights of whole bean, cotyledon and coat were lower than dry seeds, while the bean dimensions except for the length were below dry seeds (1.51 vs. 1.3 cm) [12]. Moisture of raw beans was higher than cooked beans and comparable to dry seeds [12]. Protein exceeds in raw over cooked beans (30.62% vs. 27.47%) indicates cooking destroys bean protein. Protein of raw and cooked beans is comparable with most edible legumes (17–30%) [1,39] and exceeds winged bean (20.6%), sword bean (26.8%), velvet bean (23.5%), green gram (22.3%), black gram (23.3%), pigeon pea (19.4%), chick pea (20.7%), cow pea (22.5%), whole wheat flour (8.5%), parboiled rice (7.7%), and egg (12.6%) [40–48]. Lipid in beans ranged between 1.92% (cooked) and 2.26% (raw), which is higher than dry seeds of *Canavalia* species (1.6–1.8%) [12,49] and lower than dry seeds of edible wild legumes of India (4.6–12.3%) [50]. Crude fiber of raw beans (10.65%) was more than cooked beans (6.98%) and dry seeds (1.7–2.26%) [12], while less than dry seeds of *Canavalia gladiata* (12.8%) and *C. maritima* (17.3%) of Central America [49]. Bressani et al. [49] indicated that high amount of seed coat of *C. maritima* dry seeds contribute to high fiber. Although low crude fiber is nutritionally appreciable as it traps less proteins and carbohydrates [51], certain health benefits have been reported by high fiber diets (e.g. lowering blood cholesterol and reducing risks of large bowel cancers) [52,53]. Ash was higher in raw (3.18%) than cooked (2.4%) beans, but below dry seeds [12] of *Canavalia ensiformis* (4.64%), and *C. gladiata* (3.72). Crude carbohydrate was higher in cooked (61.23%) than raw (53.29%) beans as seen in dry seeds [12]. High carbohydrate in seeds known to combat intestinal cancers [54] and characterized by low glycemic index, which helps in the prevention and management of type II diabetes mellitus [55]. The energy of beans elevated on cooking as in dry seeds [12]. Vita-

Table 8
Mean food intake, protein intake, gain in body weight, FER, NPR, PRE, TD, BV and NPU of casein and ripened bean flour diets of *Canavalia maritima* fed to rats.

Assay	Casein*	Ripened beans*	
		Raw	Cooked
Growth studies			
Food intake for 28 d (g)	144.31 ± 2.4^{a}	$90.06 \pm 0.6^{b,c}$	$117.74 \pm 1.72^{b,d}$
Protein intake for 28 d (g)	14.39 ± 0.23^{a}	$9.06 \pm 0.06^{b,c}$	$11.77 \pm 0.17^{b,d}$
Gain in body weight for 28 d (g)	34.88 ± 0.42^{a}	$1.47 \pm 0.3^{b,c}$	$6.10 \pm 0.05^{b,d}$
FER	0.24 ± 0.01^{a}	$0.02 \pm 0.004^{b,c}$	$0.06 \pm 0.001^{b,d}$
PER	2.42 ± 0.06^{a}	$0.17 \pm 0.04^{b,c}$	$0.54 \pm 0.007^{b,d}$
Corrected PER**	2.50 ± 0^{a}	$0.17 \pm 0.03^{b,c}$	$0.56 \pm 0.007^{b,d}$
Gain in weight for 10 d (g)	9.63 ± 0.17^{a}	$0.56 \pm 0.005^{b,c}$	$2.19 \pm 0.007^{b,d}$
Weight loss for 10 d (g)	2.64 ± 0.03	2.64 ± 0.03	2.64 ± 0.03
Protein consumed for 10 d (g)	4.73 ± 0.04^{a}	$2.97 \pm 0.12^{b,c}$	$3.93 \pm 0.06^{b,d}$
NPR	2.59 ± 0.02^{a}	$1.08 \pm 0.06^{b,c}$	$1.24 \pm 0.03^{b,d}$
PRE	41.51 ± 0.41^{a}	$17.21 \pm 1.01^{b,c}$	$19.80 \pm 0.54^{b,d}$
Nitrogen balance studies			
TD(%)	98.50 ± 0.37^{a}	$41.76 \pm 3.27^{b,c}$	$53.33 \pm 2.15^{b,d}$
BV(%)	86.18 ± 0.24^{a}	$30.40 \pm 2.3^{b,c}$	$40.20 \pm 0.46^{b,c}$
NPU(%)	85.73 ± 0.47^{a}	$12.71 \pm 0.92^{b,c}$	$21.42 \pm 1.02^{b,d}$

* Values (mean $\pm$ SD, $n = 5$) across the columns with different letters are significantly different ($P < 0.05$, t test).
** Based on values of 2.5 as standard for casein.

min C was higher in raw than cooked beans (0.23 vs. 0.07 mg/100 g), but lower than green gram, bengal gram and horse gram (2.4–3.9 mg/100 g) [56].

The beans are rich in potassium, magnesium, zinc and manganese and they meet the National Research Council/National Academy of Sciences (NRC/NAS) recommended pattern for infants [57]. Iron, zinc and manganese were lower in ripened beans than dry seeds [12]. A bean diet with low sodium is suitable for individuals suffering with hypertension. Selenium, being prosthetic group of antioxidant enzymes, protects cells against free radicals [58] and also known to prevent the toxic effects of heavy metals (e.g. arsenic, cadmium, mercury and tin).

Total proteins, albumins, globulins and glutelins were higher in raw beans than cooked beans and lower than dry seeds [12]. Prolamins were higher in beans (0.28 vs. 0.55–0.74%) than dry seeds [12]. The SDS-PAGE of raw beans revealed four major protein fractions (53.7, 40.36, 34.1 and 16.49 kDa), while three protein fractions in cooked beans (48.59, 26.96 and 9.86 kDa) with smear like unclear bands indicating partial denaturation. Albumins are known to contain more sulfur-amino acids and other EAA [59]. True protein (27%) of raw beans surpassed winged bean (15.2%), sword bean (20.8%) and *Cassia floribunda* (16–17.7%) [40,60]. Canavanine (2-amino-4-guanidinooxy-butyric acid), an antimetabolite, reported about 3% and 5% of seed dry matter of *C. ensiformis* and *Canavalia brasiliensis* [61,62] respectively.

Glutamic acid (10.5–19.1 vs. 8.1–18%) and tyrosine (0.2–10.31 vs. 0.19–4%) in ripened beans exceeded the concentrations in dry seeds [12]. The EAAs (threonine, valine, isoleucine, leucine, tyrosine/phenylanine and lysine) in raw and cooked ripened beans fulfill the FAO/WHO/UNU pattern of requirement for adults [38]. Generally, legumes are high in lysine and low in sulfur-amino acids [63]. Although, lysine is lower in beans than in dry seeds [12], a considerable amount of lysine (4.22–4.26%) exists in raw as well as cooked beans, which surpassed the FAO/WHO/UNU recommended pattern for adults [38].

The total quantity of saturated and unsaturated fatty acids is lower in beans (Table 6) than dry seeds [12]. The sum of the essential fatty acids in cooked beans was more than cooked dry seeds (7.3 vs. 0.4 mg/g lipid) [13]. Cooking increased the essential fatty acid, linolenic acid (0 vs. 7.3 mg/g lipid) in beans unlike in cooked dry seeds [12]. Linoleic as well as arachidonic acids confined to raw beans or raw as well as cooked dry seeds [12,13]. The polyunsaturated fatty acids/saturated fatty acids (P/S) ratio was elevated in raw beans than cooked beans, while reverse in dry seeds [12,13]. As the beans of *C. maritima* possess low fat protein, it may be suitable to combat protein-energy malnutrition particularly in hyperlipedemic patients [64].

Total phenolics decreased to about 50% in beans on cooking, but it was lower in raw and cooked dry seeds [12,13] than beans. Hemagglutinating activity is due to the presence of lectins (e.g. con A), which combines with cells lining the intestinal mucosa and interferes with absorption of nutrients [65]. In dry seeds of *C. ensiformis*, lectin constitutes 20% of total protein [66]. Although *C. maritima* beans are potential source of protein, their bioavailability becomes limited probably due to presence of con A. A considerable decrease in antinutritional factors of beans on cooking indicates further scope for different thermal treatments to eliminate or reduce to below threshold level.

Food intake of cooked beans by rats was better than raw beans (117.74 vs. 90.06 g), which is similar to dry seeds [12]. The gain in body weight of rats feeding on raw and cooked bean diets was comparable with dry seeds [12], but lower than casein diet. However, feeding cooked bean or cooked dry seed diets [13] resulted elevated body weight in rats than raw bean or raw seed diets [12]. Low food intake, decreased FER as well as PER and other growth parameters in cooked beans as seen in cooked dry seed diet [13]. The NPR and PRE of cooked beans were better than raw beans, but lower than casein. Feeding cooked bean diet resulted in higher TD, BV and NPU than raw bean diet corroborating the results of earlier studies on dry seed diet [12,13]. Bressani and Sosa [67] also showed that feeding rats with roasted whole seed diet of *C. ensiformis* decreased the weight. An important antinutritional factor limits the use of *Canavalia* beans as food is con A-like lectin, which may have resulted in low TD, BV and NPU in our study. Con A is known to trigger over production of mucus resulting in high fecal output in rats fed with raw seed diets. Even though feeding cooked beans improved the nitrogen balance with respect to raw beans in rats, the nitrogen balance was insufficient, possibly due to the residual effect of toxin in the cooked bean diet. In spite of low NPU by antinutritional factors, the animals receiving raw bean or cooked bean diets showed a positive nitrogen balance, suggesting further scope to eliminate toxins by improved thermal processing strategies. Although cooked ripened beans of *C. maritima* fulfils the recommended nutritional values, it is necessary to confirm whether there are any intestinal abnormalities in the rats fed with such diets.

5. Conclusions

Raw and pressure-cooked *C. maritima* ripened beans possess high proximal value (protein, carbohydrate, fiber, energy) and minerals with low lipids. Globulins and albumins are comparable to common edible legumes. Many essential amino acids (threonine, valine, isoleucine, leucine, tyrosine/phenylalanine and lysine) in raw as well as cooked beans satisfy the FAO/WHO/UNU pattern of requirement for adults. Linolenic acid is highest in cooked beans, while linoleic and arachidonic acids are confined to raw beans. Total phenolics and tannins in beans are low and devoid of orthodihydric phenols as well as trypsin inhibitors. The hemagglutinating activity of beans considerably decreased on cooking. Positive nitrogen balance in rats received cooked bean diet gives a clue for possible enhancement of nutritional value of ripened beans of *C. maritima* through alternative thermal strategies.

Acknowledgements

Authors are grateful to Mangalore University for granting permission to carry out this study at the Department of Biosciences. Thanks are due to K.K. Vijayalaxmi, Department of Applied Zoology for helpful suggestions.

References

[1] N.R. Reddy, M.D. Pierson, D.K. Salunkhe, Legume Based Fermented Foods, CRS Press Inc., Boca Raton, Florida, 1986.

[2] B. Becker, Wild plants for human nutrition in the sahelian zone, J. Arid. Environ. 11 (1986) 61–64.

[3] G.I.O. Badifu, Food potential of some unconventional oilseeds grown in Nigeria – A brief review, Pl. Foods Hum. Nutr. 43 (1994) 211–224.

[4] D.F. Apata, A.D. Ologhobo, Biochemical evaluation of some Nigerian legume seeds, Food Chem. 49 (1994) 333–338.

[5] F.N. Madubuike, P.C. Ojimelukwe, P.O. Ajah, Proximate composition, energy content and physicochemical properties of *Afzelia africana* and *Brachystegia eurycoma* seeds, Pl. Foods Hum. Nutr. 46 (1994) 339–344.

[6] I.E. Ezeagu, C.C. Metges, J. Proll, K.J. Petzke, A.O. Akinsoyinu, Chemical composition and nutritive value of some wild-gathered tropical plant seeds, Food Nutr. Bull. 17 (1996) 275–278.

[7] K.J. Petzke, I.E. Ezeagu, J. Proll, A.O. Akinsoyinu, C.C. Metges, Amino acid composition, available lysine content and in vitro protein digestibility of selected tropical crop seeds, Pl. Foods Hum. Nutr. 50 (1997) 151–162.

[8] R.K. Arora, K.P.S. Chandel, B.S. Joshi, K.C. Pant, Rice bean: Tribal pulse of eastern India, Econ. Bot. 34 (1980) 260–263.

[9] N. Gunjatkar, V.D. Vartak, Enumeration of wild legumes from Pune district, Maharashtra, J. Econ. Taxon. Bot. 3 (1982) 1–9.

[10] J. Smartt, Grain Legumes, Cambridge University Press, Cambridge, 1990.

[11] A.B. Arun, K.R. Sridhar, N.S. Raviraja, E. Schmidt, K. Jung, Nutritional and antinutritional components of seeds of *Canavalia* spp. from the west coast sand dunes of India, Pl. Foods Hum. Nutr. 58 (2003) 1–13.

[12] S. Seena, K.R. Sridhar, B. Bhagya, Biochemical and Biological evaluation of an unconventional legume, *Canavalia maritima*

of coastal sand dunes of India, Trop. Subtrop. Agroecosyst. 5 (2005) 1–14.
[13] S. Seena, K.R. Sridhar, S.R. Ramesh, Nutritional and protein quality evaluation of thermally treated seeds of *Canavalia maritima* in the rat, Nutr. Res. 25 (2005) 587–596.
[14] E.C. Humphries, Mineral composition and ash analysis, in: K. Peach, M.V. Tracey (Eds.), Modern Methods of Plant Analysis, Springer, Berlin, 1956, pp. 468–502.
[15] AOAC, Official Methods of Analysis, Association of Official Analytical Chemists, Washington, DC, 1990.
[16] H.G. Müller, G. Tobin, Nutrition and Food Processing, Croom Helm Ltd., London, 1980.
[17] D.R. Osborne, P. Voogt, The Analysis of Nutrients in Food, Academic Press, New York, 1978.
[18] J.H. Roe, Chemical determination of ascorbic, dehydroascorbic and diketogluconic acids, in: D. Glick (Ed.), Methods of Biochemical Analysis, vol. 1, Interscience Publishers, New York, 1954, pp. 115–139.
[19] APHA, Standard Methods for Examination of Water and Waste Water, American Public Health Association, USA, 1995.
[20] S.M.M. Basha, J.P. Cherry, C.T. Young, Changes in free amino acid, carbohydrates and proteins of maturing seeds from various peanut (*Arachis hypogaea* L.) cultivars, Cereal Chem. 53 (1976) 586–597.
[21] J. Miersch, G. Kullertz, H. Henning, Protein phosphorylation in polysomes of pumpkin cotyledons after coumarin treatment, Bot. Acta 111 (1998) 316–324.
[22] U.K. Laemmli, Cleavage of structure proteins during assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[23] D. Hofmann, K. Jung, J. Bender, M. Gehre, G. Schüürmann, Using natural isotope variations of nitrogen in plants an early indicator of air pollution stress, J. Mass Spectro. 32 (1997) 855–863.
[24] D. Hofmann, M. Gehre, K. Jung, Sample preparation techniques for the determination of natural $^{15}N/^{14}N$ variations in amino acids by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), Isotopes Environ. Health Stud. 39 (2003) 233–244.
[25] W.A. Brand, A.R. Tegtmeyer, A. Hilkert, Compound-specific isotope analysis extending towards $^{15}N/^{14}N$ and $^{13}C/^{12}C$, Org. Geochem. 21 (1994) 585–594.
[26] R. Garces, M. Mancha, One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues, Anal. Biochem. 211 (1993) 139–143.
[27] J. Rosset, F. Bärlocher, J.J. Oertli, Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams, Int. Rev. Gesamten Hydrobiol. 67 (1982) 695–711.
[28] R. Burns, Method for estimation of tannin in grain sorghum, Agron. J. 63 (1971) 511–512.
[29] A. Mahadevan, Biochemistry of infection and resistance, Phytopath. Z. 57 (1966) 96–99.
[30] M.L. Kakade, J.J. Rackis, J.E. McGhee, G. Puski, Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure, Cereal Chem. 51 (1974) 376–382.
[31] C.N. Hankins, J.I. Kindinger, L.M. Shannon, Legume alpha-galactosidases which have hemagglutinin properties, Plant Physiol. 65 (1980) 618–622.
[32] L.K. Gorden, A reliable method for repetitively bleeding rabbits from the central artery of the ear, J. Immun. Meth. 44 (1981) 241–245.
[33] P.L. Pellet, V.R. Young, Nutritional Evaluation of Protein Foods. Food and Nutrition Bulletin (Suppl. 4), United Nations University, 1980.
[34] A.E. Bender, B.H. Doel, Biological evaluation of protein – a new aspect, Br. J. Nutr. 1 (1957) 140–148.
[35] N. Chick, J.C.D. Hutchinson, H.M. Jackson, The biological value of protein VI. Further investigation of balance sheet method, Biochem. J. 29 (1935) 1702–1711.
[36] B.S. Platt, D.S. Miller, P.R. Payre, Protein values of human foods, in: J.F. Brock (Ed.), Recent Advance in Human Nutrition, Little Brown and Co., Boston, 1961, pp. 351–358.
[37] Stat Soft Inc., STATISTICA for Windows, Tulsa, Oklahoma, USA, 1995.
[38] FAO/WHO/UNU, Energy and protein requirements, WHO Technical Report Series # 724, Geneva, 1985.
[39] Z. Rehman, W.H. Shah, Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes, Food Chem. 91 (2005) 327–331.
[40] M.B. Viswanathan, D. Thangadurai, N. Ramesh, Biochemical valuation of *Neonotonia wightii* (Wight and Arn.) Lackey (Fabaceae), Food Chem. 75 (2001) 275–279.
[41] S. Ekanayake, E.R. Jansz, B.M. Nair, Proximate composition, mineral and amino acid content of mature *Canavalia gladiata* seeds, Food Chem. 66 (1999) 115–119.
[42] B.W. Abbey, G.O. Ibeh, Functional properties of raw and heat processed brown bean (*Canavalia rosea* DC.) flour, J. Food Sci. 52 (1987) 406–408.
[43] V.R. Mohan, K. Janardhanan, Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna*, Food Chem. 52 (1995) 275–280.
[44] C.N. Gupta, D.S. Wagle, Proximate composition and nutritive value of *Phaseolus mungoreus*. A cross between *Phaseolus mungo* and *Phaseolus aureus*, J. Food Sci. Technol. 15 (1978) 34–35.
[45] E. Nwokolo, Nutritional evaluation of pigeon pea meal, Pl. Foods Hum. Nutr. 37 (1987) 283–290.
[46] R. Jambunathan, U. Singh, Studies on Desi and Kabuli chickpea (*Cicer arietinum*) cultivars. 1. Chemical composition, in: Proceedings of the International Workshop on Chickpea Improvement, ICRISAT, Hyderabad, India, 1980, pp. 61–66.
[47] E. Nwokolo, D.I.M. Oji, Variation in metabolizable energy content of raw or autoclaved white and brown varieties of three tropical grain legumes, Anim. Food. Sci. Technol. 13 (1985) 141–146.
[48] S. Livsmedelsverk, Energi och näringsämnen, The Swedish Food Administration, Sweden, 1988.
[49] R. Bressani, R.S. Brenes, A. Gracia, L.G. Elias, Chemical composition, amino acid content and protein quality of *Canavalia* spp. seeds, J. Sci. Food Agric. 40 (1987) 17–23.
[50] V. Arinathan, V.R. Mohan, A.J. De Britto, Chemical composition of certain tribal pulses in South India, Int. J. Food Sci. Nutr. 54 (2003) 209–217.
[51] A.M. Balogun, B.L. Fetuga, Chemical composition of some under-exploited leguminous crop seeds in Nigeria, J. Agric. Food Chem. 34 (1986) 189–192.
[52] J.W. Anderson, B.M. Johnstone, M.E. Cook-Newell, Meta-analysis of the effects of soy protein intake on serum lipids, N. Engl. J. Med. 333 (1995) 276–282.
[53] J. Salvin, D.R. Jacobs, L. Marquart, Whole grain consumption and chronic disease: Protective mechanisms, Nutr. Cancer 27 (1997) 14–21.
[54] P. Aranda, J. Dostalova, J. Frias, M. Lopez-Jurado, H. Kozlowska, J. Pokorny, G. Urbano, C. Vidal-Valverde, Z. Zdyunczyk,

Nutrition, in: C.L. Hedley (Ed.), Carbohydrates in Grain Legume Seeds-Improving Nutritional Quality and Agronomic Characteristics, CAB International, Wallingford, UK, 2001, pp. 61–87.

[55] B.J. Venn, J.I. Mann, Cereal grains, legumes and diabetes, Eur. J. Clin. Nutr. 58 (2004) 1443–1461.

[56] K. Naveeda, P. Jamuna, Nutrient retention in microwave cooked germinated legumes, Food Chem. 97 (2005) 115–121.

[57] NRC/NAS, Recommended Dietary Allowances, 10 edition, National Academy Press, Washington, DC, 1989.

[58] G.F. Combs, W.P. Gray, Chemopreventive agents: selenium, Pharmacol. Therapeut. 79 (1998) 179–192.

[59] J.P. Baudoin, A. Maquet, Improvement of protein and amino acid content in seeds of food legumes – a case study in *Phaseolus*, Biotech. Agronom. Soc. Environ. 3 (1999) 220–224.

[60] V. Vadivel, K. Janardhanan, Nutritional and anti-nutritional attributers of the under-utilized legume, *Cassia floribunda*, Cav. Food Chem. 73 (2001) 209–215.

[61] J.C. Gomes, M. Epstein, L.M. Maffia, A.R. Sant, Composicao quimica de sementes do feijao-bravo e de seu isolado proteico, Arq. Biol. Technol. 31 (1988) 443–459.

[62] G.A. Rosenthal, Investigation of *Canavalia*. Biochemistry in the jackbean plant *Canavalia ensiformis*. II. Canavanin biosynthesis in the developing plant, Pl. Physiol. 50 (1972) 328–331.

[63] G. Norton, F.A. Bliss, R. Bressani, Biochemical and nutritional attributes of grain legumes, in: R.J. Summerfield, E.H. Roberts (Eds.), Grain Legume Crops, Collins, London, 1985, pp. 73–114.

[64] R.N. Tharanathan, S. Mahadevamma, Grain legumes a boon to human nutrition, Trends Food Sci. Technol. 14 (2003) 507–518.

[65] I. Liener, Heat-labile antinutritional factors, in: J. Summerfield, A.H. Bunting (Eds.), Advances in Legume Science, Royal Botanic Gardens, Kew, 1980, pp. 151–170.

[66] K. Dalkin, D.J. Bowles, Analysis of inter-relationship of jack bean and seed components by two-dimensional mapping of iodinated tryptic peptides, Planta 157 (1983) 536–539.

[67] R. Bressani, J.L. Sosa, Effect of processing on the nutritive value of *Canavalia* Jack beans (*Canavalia ensiformis* L.), Pl. Foods Hum. Nutr. 40 (1990) 207–214.